

# Collection of Genomic DNA by Buccal Swabs for Polymerase Chain Reaction-Based Biomarker Assays

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Studies in molecular and genetic epidemiology require a high-throughput, low cost, and reliable means of genomic DNA collection. Buccal (cheek) swabs have been proposed as a means of achieving these goals, but there is little information about the practical application of this approach. From January 1995 to December 1997, we processed 995 buccal swabs for use in polymerase chain reaction (PCR)-based genotype assays in the context of ongoing molecular epidemiologic studies. Six hundred forty-seven of these swabs were processed immediately after collection and 348 were received by mail. We were able to obtain at least one genotype from 99.7% (645 of 647) of fresh-processed and 97.4% (330 of 339) of mailed biosamples. A PCR success rate of 90.3% (2,546 genotypes from 2,819 assays) was achieved. Genotypes were obtained from 96.1% (1,865 genotypes from 1,941 assays) of fresh-processed biosamples and 77.6% (681 genotypes from 878 assays) of mailed biosamples. PCR success rates at any single locus ranged from 92.6 to 98.8% (fresh-processed) and 75.5 to 79.6% (mailed). The PCR success rate among fresh-processed biosamples was significantly higher than among mailed biosamples (Fisher's exact test  $p < 0.0001$ ), and more attempts were required to obtain a successful PCR result for mailed biosamples as compared to fresh-processed biosamples. For one locus (*CYP3A4*), a subset of mailed biosamples was purified if two or more PCR failures occurred. Additional genotypes were obtained in 58.3% of these previously failed biosamples. Time from biosample receipt to DNA extraction had no effect on PCR success. After storage of processed biosamples for as long as 3 years, there was no appreciable decrease in the rate of PCR success. These results suggest that adequate DNA for PCR-based applications can be obtained from buccal swabs, but sampling or processing considerations may be important in obtaining optimal results. **Key words:** biosample, genetic epidemiology, molecular epidemiology, polymerase chain reaction. *Environ Health Perspect* 107:517–520 (1999). [Online 12 May 1999] <http://ehpnet1.niehs.nih.gov/docs/1999/107p517-520walker/abstract.html>

Numerous methods are available for the collection of genomic DNA for use in molecular epidemiologic and other studies that require high-throughput, reliable biosampling methods. These methods include the collection of peripheral blood by venipuncture or on blood spot (Guthrie) cards (1); hair roots (2); urine (3–6); mouth washes (7); and wood, brush, or cotton buccal swabs (8–13). Each approach has distinct advantages and disadvantages. Venipuncture collection of peripheral blood is the most expensive and most difficult approach to process. It is also invasive and confers the most discomfort to study subjects and the greatest risk of infection to laboratory personnel. However, it also provides the material required for protocols such as Southern blotting or initiation of cell lines. In contrast, other available methods are less invasive and less costly, but may provide smaller or inconsistent quantities of DNA. These approaches may be appropriate for some molecular epidemiologic applications in which high-throughput processing capability is required. In the present study, we evaluated the use of buccal swab biosampling to obtain genomic DNA for use in polymerase chain reaction (PCR)-based genotyping assays.

## Materials and Methods

### Sample subjects and biosample collection.

Biosamples were obtained from 995 study subjects between January 1995 and December 1997. Study subjects were participants in molecular epidemiologic studies of prostate cancer and melanoma at the Hospital of the University of Pennsylvania (HUP). These subjects included 447 cases of prostate cancer, 166 cases of melanoma, and 382 controls. These subjects ranged in age from 40 to 96 years (mean, 62.7 years). All subjects were contacted through outpatient clinics at HUP, and provided informed consent under institutional review board-approved protocols. These biosamples were collected from buccal swab (cytological) brushes (Cyto-Pak Cytosoft Brush, Camarillo, CA), which were provided to each subject in sealed, sterile plastic tubes. Subjects were given the choice to collect the swab at the time of their clinic visit or take the swab with them to be collected and mailed back at a later date. All subjects were provided with an instruction card to which the buccal swabs were attached. The card guided the subjects to remove the swab from the sterile tube and rub the brush on the inside of the

mouth for 15–30 sec. The subjects were then instructed to replace the swab in the tube and either return it immediately to study staff or mail it back using an attached stamped envelope. Six hundred forty-seven (65%) subjects agreed to participate immediately by self-collecting and returning a buccal swab biosample at the time of their clinic visit. The remaining 348 (35%) subjects chose to self-collect the buccal swab biosample after the time of their clinic visit, or were contacted for participation by mail after the time of their clinic visit. The latter group of subjects returned the swabs to the laboratory by U.S. mail.

Although it was outside the scope of the present study to evaluate the acceptability of buccal swab sampling overall or in comparison with venous blood sampling, we achieved a 95% participation rate overall among study subjects who agreed to participate in our studies to date (data not shown), with 85–90% participation among those who mailed back buccal swabs and nearly 100% among those fresh-processed. When recontacted, many of those subjects who did not return the mailed buccal swabs cited having been very busy or having forgotten to return the swab. Although not based on systematic data collection, these anecdotal results suggest that the level of acceptance of buccal swabs for biosample collection may be high.

**Biosample processing.** Upon receipt in the laboratory, all swabs were placed at 4°C until DNA extraction. Genomic DNA extraction was undertaken using a protocol modified from Richards et al. (11). Upon receipt in the laboratory and after removal of the swab handle, the brush section of the swab was placed inside a 1.5-mL microcentrifuge tube, and 600  $\mu$ L of 50 mM NaOH was added. The tube containing the brush

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was closed and then vortexed for 10 min. The tube was then heated at 95°C for 10 min. Finally, 120  $\mu$ L 1 M Tris (pH 8.0) was added to the tube, after which the brush was removed and discarded. The resulting biosample solution containing genomic DNA was stored at 4°C. Between 5 and 10  $\mu$ L of this biosample is used in a typical PCR reaction, suggesting that the 720  $\mu$ L biosample should provide sufficient genomic DNA for 72–144 PCR reactions. A randomly chosen subset of buccal swab biosamples was purified if the sample produced two or more failed PCR assays at *CYP3A4* (whether or not they failed in any other assay). Purification was undertaken using the Puregene DNA Isolation Kit D5000A (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's recommended protocol. All processed biosamples were stored at 4°C from time of DNA extraction until PCR analysis.

**PCR genotype analysis.** The protocol of Rebbeck et al. (14) was used to amplify a 592-bp fragment upstream from *CYP3A4*. Amplification products were visualized by conformation-sensitive gel electrophoresis of the PCR product on a 10% nondenaturing polyacrylamide gel after staining with ethidium bromide. *CYP3A4* genotyping was not undertaken on melanoma cases.

Glutathione-S-transferase  $\mu$  (*GSTM1*) genotypes were generated using a protocol modified from Davies et al. (15). The primers amplifying *GSTM1* were 5' CTG CCC TAC TTG ATT GAT GGG 3' and 5' CTG GAT TGT AGC AGA TCA TGC 3'. As a positive internal control, a portion of the  $\beta$ -globin gene was amplified simultaneously with *GSTM1* using the primers of Saiki et al. (16). The PCR reagent mix consisted of 5  $\mu$ L 10 $\times$  reaction buffer [100 mM Tris-HCl (pH 9.0), 500 mM KCl, 1% (v/v) Triton, 15 mM MgCl<sub>2</sub>], 1  $\mu$ L 10 mM dNTPs, 5  $\mu$ L each 5- $\mu$ M primers, 10  $\mu$ L buccal swab DNA, 14.5  $\mu$ L dd H<sub>2</sub>O, and 0.5  $\mu$ L AmpliTaq (Perkin Elmer, Norwalk, CT), for a total volume of 51  $\mu$ L. The temperature cycling protocol was one cycle of 94°C for 3 min, 82°C for 1 min, then 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72° for 2 min, followed by one cycle for 10 min at 72°C. Glutathione-S-transferase  $\theta$  (*GSTT1*) genotypes were generated using the primers of Pemble et al. (17). As with *GSTM1*, simultaneous amplification of the  $\beta$ -globin gene (16) was used as a positive internal control. The PCR reaction mixture consisted of 0.25  $\mu$ L 10 mg/mL bovine serum albumin, 2.5  $\mu$ L 10 $\times$  PE buffer II (Perkin Elmer), 1  $\mu$ L 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ L 10 mM dNTPs, 2.5  $\mu$ L of each 5- $\mu$ M primers, 6  $\mu$ L buccal swab DNA, 4.45  $\mu$ L dd H<sub>2</sub>O, and 0.3  $\mu$ L AmpliTaq, for a total

volume of 25  $\mu$ L. The temperature profile for the PCR reaction was 93°C for 2.3 min, 82°C for 1 min, 35 cycles of 93°C for 1 min, 58°C for 1 min, and 72°C for 1 min, followed by a cycle of 93°C for 30 sec, 58°C for 1 min, and 72°C for 7.5 min. For both *GSTM1* and *GSTT1*, visualization took place on a 2% agarose gel after staining with ethidium bromide. PCR success was defined as any scorable genotype result. *GSTM1* or *GSTT1* genotypes were not considered valid unless a successful  $\beta$ -globin amplification product was observed.

**Statistical methods.** Comparisons of proportions in contingency tables used Fisher's exact tests (FET). Kruskal-Wallis  $\chi^2$  statistics were used for analysis of continuous variables. All reported *p*-values were based on two-sided tests.

## Results and Discussion

**Effect of fresh-processed versus mailed biosampling.** Table 1 presents a comparison of PCR-based genotype results on mailed and fresh-processed buccal swabs. PCR failure occurred at all three test loci in only 12 of 995 subjects. Thus, we estimated a 98.8% PCR success rate overall, with a success rate of 99.7% in fresh-processed and 97.4% in mailed biosamples. Of the 2,819 assays undertaken at three loci, successes were observed in 2,546 (90.3%) assays. Of the 1,941 genotype assays undertaken in fresh-processed biosamples, PCR successes were observed in 1,865 (96.1%) assays. These estimates are consistent with those reported by Richards et al. (11), who reported a PCR success rate of 95 and 99% at the *CFTR* gene after collecting one and two buccal swabs, respectively. In contrast, PCR success among mailed biosamples occurred in 681 of 878 (77.6%) assays. The success rate in fresh-processed biosamples was significantly higher than that in mailed biosamples (FET *p*-value < 0.0001). As indicated in Table 1, PCR success rates at any single locus ranged from 92.6 to 98.8% for fresh-processed samples and 75.3 to 81.3% for mailed samples. Although not

systematically evaluated, the majority of those samples on which we obtained PCR results were achieved on the first attempt. However, the present study was limited because we were not able to systematically quantitate the distribution of repeat assays required to obtain a PCR success. Despite this limitation, we conclude that the majority of both fresh-processed and mailed buccal swabs can provide adequate DNA for PCR analysis. However, fresh-processed buccal swab biosamples are less likely to have PCR failures than mailed biosamples using the protocols described here. Thus, the higher PCR failure rate among mailed biosamples could be explained by decreased DNA quality or quantity rather than the total absence of DNA.

We also observed significant differences in PCR success rates across loci (Table 1). Fresh-processed biosample PCR success rates were significantly different between *GSTM1* and *GSTT1* (FET *p*-value = 0.034), between *GSTM1* and *CYP3A4* (FET *p*-value < 0.0001), and between *GSTT1* and *CYP3A4* (FET *p*-value = 0.0003). Mailed biosample PCR success rates were not significantly different across loci. Therefore, there may be substantial variation in PCR success rates depending on the locus or assay used. Although each of the assays used was optimized for buccal swab biosamples, it may be useful to consider optimizing PCR conditions depending on the biosample collection method. For example, the use of NaOH in DNA extraction may alter the pH of the PCR reaction, even though a small quantity of buccal swab-derived biosample is used. Thus, optimization of PCR conditions considering pH may be an important step in determining PCR conditions. However, we were able to achieve high rates of PCR success after we optimized amplification conditions for these buccal swab biosamples. Our results suggest that the potentially high pH of our processing/storage solution need not adversely affect the ability to obtain high PCR success rates.

**Table 1.** Comparison of PCR results using fresh-processed and mailed buccal swabs: number of assay successes/total assays conducted (percent success).<sup>a</sup>

	<i>GSTM1</i>	<i>GSTT1</i>	Unpurified <sup>b</sup>	<i>CYP3A4</i> purified <sup>b</sup>	Total	<i>GSTM1</i> , <i>GSTT1</i> , and <i>CYP3A4</i>
Fresh-processed	639/647 (98.8%)	627/647 (96.9%)	509/511 (99.6%)	90/136 (66.2%)	599/647 (92.6%)	1,865/1,941 (96.1%)
Mailed	271/348 (77.9%)	262/348 (75.3%)	120/134 (89.6%)	28/48 (58.3%)	148/182 (81.3%)	681/878 (77.6%)
Total	910/995 (91.5%)	889/995 (89.3%)	629/645 (97.5%)	118/184 (64.1%)	747/829 (90.1%)	2,546/2,819 (90.3%)

Abbreviations: *GSTM1*, glutathione-S-transferase  $\mu$ ; *GSTT1*, glutathione-S-transferase  $\theta$ ; PCR, polymerase chain reaction.

<sup>a</sup>In all comparisons of success rates in fresh-processed versus mailed buccal swabs, fresh-processed biosamples had a significantly higher PCR success rate with a Fisher's exact test *p*-value < 0.0001. <sup>b</sup>Purified samples were those that had failed on initial analysis and were then subjected to a process that removed non-DNA contaminants. Nonpurified samples were not subjected to this process.

Finally, multiple attempts were sometimes necessary before a PCR success was obtained, and the number of repeat attempts required to achieve PCR success varied significantly between fresh-processed and mailed biosamples. We collected information about the number of attempts required to achieve PCR success for 825 *CYP3A4* genotypes and 990 *GSTM1* genotypes. For *CYP3A4*, 616 (74.7%) PCR reactions were successful on the first attempt, whereas 97 (11.8%) required two attempts, 67 (8.1%) required three attempts, and 45 (5.5%) required four or more attempts. The mean number of PCR attempts before a success at *CYP3A4* was 1.3 [standard deviation (SD) = 0.8] for fresh-processed biosamples and 1.8 (SD = 1.3) for mailed biosamples [Kruskal-Wallis  $\chi^2 = 16.49$ , degrees of freedom (*df*) = 1, *p*-value < 0.0001]. For *GSTM1*, 655 (66.2%) PCR reactions were successful on the first attempt, 102 (10.3%) required two attempts, 137 (13.8%) required three attempts, and 96 (9.7%) required four or more attempts. The mean number of PCR attempts before a success at *GSTM1* was 1.5 (SD = 1.0) for fresh-processed biosamples and 2.1 (SD = 1.3) for mailed biosamples (Kruskal-Wallis  $\chi^2 = 82.40$ , *df* = 1, *p*-value < 0.0001). Therefore, the majority of biosamples provided a successful PCR result after a single attempt, but the number of attempts required to achieve PCR success was significantly higher in mailed biosamples as compared to fresh-processed biosamples.

Our data do not allow us to directly infer why PCR failures were more common among mailed biosamples. One explanation for the observed differences is incorrect biosample collection by subjects, who did not collect the biosamples in the presence of study staff. It is plausible that some mailed swabs contained insufficient material because of shorter or less vigorous swabbing than might have occurred in the clinic. Buccal swabs were accompanied by an instruction card that described the proper swabbing procedure. However, there was no way to evaluate whether the subjects adhered to the recommended collection method. The vigorous processing that included extreme temperature and pH conditions minimized the possibility of bacterial contamination of the samples. This was supported by the lack of apparent bacterial growth in any biosample. Another explanation for increased mailed biosample failures includes exposure to extremes in temperature or drying out of the swabs prior to their arrival at the laboratory. Upon receipt, we observed a small number of damaged (e.g., cracked or shattered) tubes that may have dried out prior to processing. However,

there was no higher failure rate among these samples that could explain a significant proportion of mailed biosample PCR failures (results not shown).

Biosample storage conditions prior to laboratory receipt could have contributed to DNA degradation or otherwise result in poor biosample quality. Meulenbelt et al. (12) suggested that cotton swabs kept dry for 7 or more days after collection and before processing resulted in decreased DNA yields. Freeman et al. (13) evaluated the use of a buffered storage/preservative solution (STE buffer: 100 mM NaCl, 10 mM Tris-HCL at pH 8, 10 mM EDTA at pH 8, 0.2 mg/mL proteinase K, and 0.5% SDS) for use in mailed buccal swab biosampling protocols. This protocol used three biosample collections per subject at various times in a day, using a total of 10 cotton swabs stored for mailing in STE buffer. At least one PCR-based genotype was obtained from all adult biosamples. Although these conditions may be optimal for mailed buccal swab protocols, the requirement of multiple swabbings may be a limiting factor for some studies. As a partial solution, we now request at least two buccal swabs from each study subject to increase the chances of obtaining sufficient DNA for PCR applications.

**Effect of biosample purification.** We evaluated whether the purification of buccal swab biosamples could provide additional PCR success for biosamples in which PCR failures occurred at *CYP3A4*. Presumably, purification increases DNA concentration and removes impurities (e.g., proteins and cell residue). A random subset of biosamples was purified because the biosamples previously failed in more than one *CYP3A4* assay. It was not surprising that the *CYP3A4* failure rate remained high even after purification (Table 1). However, additional *CYP3A4* genotype data were obtained from 58.3% of all purified biosamples, which increased the PCR success rate from 65.9% (120 PCR successes of 182 biosamples prior to purification) to 81.3% for *CYP3A4* overall. Furthermore, PCR failures were not significantly different

between purified fresh-processed and purified mailed buccal swab biosamples (FET *p*-value = 0.382). These results suggest that purification of mailed biosamples may improve PCR success.

**Effect of time from laboratory receipt to DNA extraction.** The mean time from laboratory receipt to DNA extraction (i.e., preprocessing storage time at 4°C) was 1.0 day overall (range, 0–9 days). Fresh-processed buccal swabs had a longer mean delay until processing than mailed buccal swabs (1.1 vs. 0.8 days; Kruskal-Wallis  $\chi^2_1 = 35.33$ , *p*-value < 0.0001), with 75.3% of fresh-processed and 83.3% of mailed buccal swabs processed within 1 day of laboratory receipt. Therefore, higher failure rates among mailed biosamples cannot be explained by longer delays from laboratory receipt until processing. There was also no clear trend toward higher *CYP3A4* PCR failure rates with longer delays between biosample receipt and processing (Table 2). For example, biosamples stored for 4 days or more before processing did not have significantly higher failure rates than those processed immediately upon receipt in the laboratory (FET *p*-value = 0.215 for fresh-processed and 0.198 for mailed biosamples). Therefore, it does not appear that immediate biosample processing is necessary to achieve good PCR results. Our experience is consistent with that reported by Richards et al. (11), who evaluated PCR success after storage of swabs at 4°C prior to processing for periods of 3 days, 1 week, 2 weeks, 1 month, and 10 months, and found no significant effect on PCR yield. These authors also reported no significant effect on DNA yield from swabs stored prior to processing in high temperature, high humidity, or freezing conditions. Our experience with mailed buccal swabs suggests that uncontrolled storage or collection conditions prior to laboratory receipt may affect PCR success. However, we have not collected data about time from actual swab collection to receipt in the laboratory for mailed samples, nor about storage conditions prior to mailing. Therefore, we cannot evaluate the effect of conditions prior

**Table 2.** Effect of time from biosample receipt to processing (i.e., preprocessing storage time) on polymerase chain reaction success.

Process delay (days)	No. successes/total biosamples analyzed (% success) <sup>a</sup>		
	Fresh-processed	Mailed	Total biosamples
0	236/258 (91.5%)	143/223 (64.1%)	379/481 (78.8%)
1	203/229 (88.6%)	39/67 (58.2%)	242/296 (81.8%)
2	26/34 (76.5%)	11/17 (64.7%)	37/51 (72.5%)
3	73/77 (94.8%)	14/16 (87.5%)	87/93 (93.5%)
4	28/33 (84.8%)	3/6 (50.0%)	31/39 (79.5%)
5–9	13/16 (81.3%)	9/19 (47.4%)	22/35 (62.9%)
Total	579/647 (89.5%)	219/348 (62.9%)	798/995 (80.2%)

<sup>a</sup>Includes any failure at glutathione-S-transferase  $\mu$  or glutathione-S-transferase  $\theta$ .

to the receipt of mailed buccal swabs in the laboratory. Additional follow-up will also be required to evaluate the effect of long-term storage on PCR success in buccal swab biosamples.

**Effect of long-term storage.** To evaluate the effect of long-term biosample storage, we retrieved 137 fresh-processed biosamples that had been stored for 12–24 months ( $n = 50$ ), 24–36 months ( $n = 50$ ), and more than 36 months ( $n = 37$ ) at 4°C. The maximum storage time was 41 months. Each of these samples was selected for reanalysis because we had obtained a successful PCR result at *GSTM1* and *GSTT1* at the time of the initial biosample processing. The *GSTM1* and *GSTT1* assays were repeated on all 137 samples. A successful PCR result was obtained for at least one locus from all samples. This result suggested that no complete degradation of DNA occurred following storage at 4°C. However, of the 274 genotype assays undertaken (i.e., 137 biosamples assayed for *GSTM1* and *GSTT1*), 24 PCR failures at one locus or the other (8.8%) were observed: 6 of 100 assays (6%) failed in samples stored 12–24 months, 11 of 100 assays (11%) failed in samples stored 24–36 months, and 7 of 74 assays (9.5%) failed in samples stored more than 36 months. This did not represent a trend toward higher failure rates with longer storage times ( $\chi^2 = 1.63$ ,  $df = 2$ ,  $p = 0.443$ ). Despite the fact that PCR success rates remained high after storage at 4°C for at least 12 months, these results imply that PCR success may diminish slightly after storage relative to genotype results obtained shortly after biosample collection.

**DNA concentration.** We estimated DNA concentration using the DNA Dipstick Kit (InVitrogen, Inc., Carlsbad, CA) using DNA biosamples from a random sample of 43 buccal swabs. As described below, half of these had immediate PCR success and the other half had repeated PCR failures. The mean DNA concentration overall was 3.7 ng/μL (SD, 3.1; range, 0.3–10.0). Because the total volume of our sample was approximately 720 μL, we estimated a mean DNA yield of 2.7 μg per buccal swab. Although this estimate was based on a small number of samples using a relatively crude quantification method, it was consistent with the report of Freeman et al. (13), who used spectrophotometry and agarose gel comparisons against standards of known DNA concentrations to

quantitate the amount of DNA obtained under their 10-swab protocol. They obtained an average yield of 32 μg (range, 3.2–110.8 μg) from 2-year-old children and 38 μg (range, <1–108 μg) from adults. Taken together, these results suggest that a single buccal swab may yield approximately 3 μg of genomic DNA. In contrast, peripheral blood samples typically produce genomic DNA concentrations in excess of 100 μg.

To evaluate whether PCR failure rates might be explained by differences in DNA concentration, we compared 21 samples that failed to produce PCR results for at least two loci (19 of which never resulted in a successful PCR amplification), and 22 which had never failed at any locus, with PCR amplification success at the first try at every locus. There was no difference in the DNA concentration between those samples that failed (mean concentration, 3.6 ng/μL; SD, 3.0; range, 0.3–10.0 ng/μL) and those that did not fail (mean concentration, 3.7 ng/μL; SD, 3.2; range, 0.3–10.0 ng/μL; Kruskal-Wallis  $\chi^2 = 0.01$ ;  $df = 1$ ;  $p$ -value = 0.930). Although the method used to quantitate DNA here is relatively crude, we can conclude that the concentration is substantially lower than that obtained typically from DNA extracted from peripheral blood, but that PCR failures are unlikely to be explained by differences in DNA concentration or yield.

## Conclusions

Buccal swabs may be an efficient, relatively noninvasive means of obtaining DNA relative to peripheral blood sampling. Buccal swab collection protocols are less costly, less time consuming, less invasive, and pose lowered risk for both the subject and laboratory personnel. However, our results suggest that fresh-processed buccal swabs provide a higher PCR success rate than mailed buccal swabs. Thus, fresh processing of buccal swabs should be undertaken when possible. For study designs in which it may be desirable to use mailed buccal swabs, steps may need to be taken to optimize PCR success. We have determined that biosample purification can improve PCR successes. However, additional modifications to the protocols for biosample collection (e.g., collection of multiple swabs and use of buffered storage media), the mailing process (e.g., use of express mail in sealed packets to prevent drying), or the PCR assay conditions could also be considered. Despite these limitations, buccal swabs can

be useful sources of genomic DNA for some PCR-based applications.

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